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Breeding evidence for Mendelian recessive transmission in an aberrant blue form of *Sasakia charonda* (Hewitson, 1863)

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Abstract During successive breeding of wild-caught larvae of *Sasakia charonda*, an aberrant blue form emerged at the F2 generation. Repeated intra-generational crossings created a pure blue line at the F5 generation. Crossing experiments between blues and normals confirmed Mendelian recessive transmission of the blue allele.

Key words blue aberrant form, mendelian recessive transmission, *Sasakia charonda*.

Introduction

Sasakia charonda (Hewitson,1863), called" ohmurasaki" in Japanese and "the great purple emperor" in English, is one of the largest of the nymphalid butterflies. It is distributed in Japan, Korea, China, and Taiwan and is univoltine, adults appearing and breeding once a year in nature. It hibernates as a larva. Adults have dark brown wings with white patches and a small red anal spot on each of the hindwings. The dorsal surface of the wings of males bears large purple patches proximally and females lack the purple luster. The geographical variation of the purple color in Japan ranges from a somewhat lighter shade in northern Japan, e.g. Hokkaido, to darker in the southwest, e.g. Kyushu. There has never been any mention in major illustrated books (Fujioka, 1975; Kawazoe, 1976; Shirôzu, 2006; Yata, 2007) or other literature related to *Sasakia charonda* in Japan (Ina, 1996; Kurita, 2007; Masui, 2008; Inomata, 2008) of a bright shining blue male form. Outside Japan, a bright blue form is known in Taiwan (Kodate *et al*., 2009), which might be an identical aberrancy to that described herein. A recent article about "Aberrant forms of *Sasakia charonda*" (Kodate *et al*., 2009) mentioned a somewhat similar aberrancy from Nagano Prefecture, involving an increased brilliancy of the purple color. When the senior author F.N. lived in Fukuoka Prefecture about 15 years ago, he encountered 1 male with shining blue-tinged purple patches (Figs 5 and 6), which was reared from a wild larva collected in the northern part of Oita Prefecture. Later, after returning to Shiga Prefecture, he heard from local collectors about a shining blue form, which they just called "blue". It was said to be extremely rare and only a few have been reared from wild overwintering larvae so far. In December, 2005, F. N. obtained 15 wild overwintering larvae of *S. charonda* from a friend and started breeding the resulting adult butterflies. In 2008, 2 bright shining blue males emerged in the F2 generation, which initiated the present study to clarify the hereditary mode of transmission of this aberrancy.

Materials and methods

Source of insects

The first 15 overwintering larvae of *Sasakia charonda* were collected from the Suzuka Mountains in Shiga Prefecture by one of the authors (N.W.) on December 10, 2005, and used for subsequent breeding experiments. An additional 30 wild overwintering larvae were collected about 5 km away from the original site by two other authors (T.Y. and K.K.) on December 15, 2010, and used for crossing experiments between the blue and normal forms.

Methods

1. Breeding sites and process of breeding

Breeding experiments were carried out by placing nylon nets around branches of the food plant, *Celtis sinensis*, in the garden of each author. Overwintering larvae awakened from hibernation in April when *C. sinensis* started budding. About 3 to 5 larvae were put in each net and pupation was awaited. Resulting pupae were kept indoors and adults emerged in cages. After emergence, males were kept in a cage for about 10 days to allow time for maturation before mating. Females were paired with males between the second and seventh days after the emergence of the latter. The hand-pairing method originally developed by Takakura (1956) and modified by the senior author Nagasaki (2011) was used to induce mating. After pairing, each female was released into a nylon net placed around a branch of the

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Table 1. Results of successive breeding of *Sasakia charonda*.
Appearance of blue aberrant form.

Cross Date paired Y/M/D	Phenotype of parents ♂ parent	Phenotype of parents ♀parent	No. of larvae overwintered	No. of larvae used for experiment	Phenotype of offspring ♂blue	Phenotype of offspring ♂purple	Phenotype of offspring ♀	Generation with sequence number Emerged Y/M
–	–	–	15*	15	0	5 [A]	5 [a]	F0 2006/6
Cross 06 2006/6/18	F0 purple[A]	F0 [a]	8	8	0	4 [B]	4 [b] [c]	F1 2007/6
Cross 07#1 2007/6/30	F1 purple[B]	F1 [b]	300	10	2 [C] [D]	1	1	F2-1 2008/6
Cross 07#2 2007/7/11	F1 purple[B]	F1 [c] f.*s.*	400	100	0	20	20 [d] [e] [f]	F2-2 2008/6-7

* Wild overwintering larvae collected in December 2005.
f.*s.*: forma *sugitanii*
[ ]: Alphabetical designation of individual adult used for crossing

food plant, and oviposition was awaited. After hatching, the larvae were kept in the same net until early December, when they started to descend to the ground for hibernation. Retrieved overwintering larvae were kept outdoors in a soil-filled pot through the winter.

2. Successive breeding until appearance of the aberrant blue form

The first 15 larvae collected from the wild were reared to adults in the senior author's garden as described above. In June, 2006, 5 normal males and 5 females emerged as the F0 generation. Of them, 1 male (identified as F0[A]) and 1 female (F0[a]) were hand-paired. The garden-reared progeny of this female overwintered as above and then 1 normal male (F1[B]) and 2 females (normal F1[b] and f. *sugitanii* F1[c]) emerged in June, 2007. Using them, we conducted 2 matings: 1 male (F1[B]) with 2 different females (F1[b] and F1[c]). The latter female was forma *sugitanii*, in which the hind wing anal spot is white instead of red. Among the F2 (third generation) offspring of these matings, we obtained the blue aberrant form (2 blue males).

3. Attempts to create a pure blue line

In order to confirm that the blue aberrant form that emerged following successive breedings is a hereditary characteristic, we conducted a series of successive inbreeding matings to create a pure blue line. Specifically, we coupled the 2 blue males with 3 females, which were half-siblings of the blue males. Subsequently, the emerged progeny were mated to each other. In doing so, we chose and tried to couple all the wing-color pattern combinations. When eventually all males originating from a single brood were the blue form, we coupled blue form males with full-sib females to confirm that the brood was a pure line. If so, all male progeny of the next generation should be the blue aberrant form.

4. Crossing experiment between pure blue line and wild normal butterflies

To estimate the mode of inheritance of the blue aberrancy, crosses between pure blue line and wild individuals with normal wing coloration were carried out. In December, 2010, 30 wild overwintering larvae were collected from close to the original habitat and reared to adults in June to July, 2011. All the emerged males had normal-colored wings. From them, 2 normal males and 2 females were selected randomly, then mated with individuals from the pure blue lines.

Results

1. Successive breeding until appearance of the aberrant blue form (Table 1)

A pair of normal-form butterflies (F0), which emerged from larvae collected at the same locality in December, 2005, were mated, and their progeny were inbred successively. In June, 2008, 3 males and 1 female emerged from Cross 07#1 as the F2 generation (numbered as F2-1). Two of the males had abnormally shining blue-colored wings. Because the female emerged before the males, it had passed its period of mating receptivity before any pairing among these specimens could be attempted. Cross 07#2 yielded normal males (numbered as F2-2).

2. Attempts to create a pure blue line (Tables 2-3)

Since 2 blue males emerged from the same brood, the senior author considered that this was not likely to be the result of independent new mutations but more probably a hereditary variant. Subsequent successive breeding was attempted in order to create a pure blue line. The 2 blue males from Cross 07#1 (F2-1 [C] and [D]) were coupled with 3 females from Cross 07#2 (F2-2 [d],[e], and [f]), making 3 pairs (Crosses 08#1-3) in Table 2.

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Table 2. Results of successive breeding of *Sasakia charonda*.
Creation of pure blue line.

Cross Date paired Y/M/D	Phenotype of parents ♂ parent	♀parent	No. of larvae overwintered	No. of larvae used for experiment	Phenotype of offspring ♂blue	♂purple	♀	Generation with sequence number Emerged Y/M
Cross 08#1 2008/7/4	F2-1 blue[C]	F2-2 [d]	2nd brood	6	0	2 [E]	4 [g]	F3-1-1 2008/9-11
	F2-1 blue[C]	F2-2 [d]	400	20	0	12	7 [j] [k] [l]	F3-1-2 2009/6-7
Cross 08#4 2008/10/10	F3-1-1 purple[E]	F3-1-1 [g]	3rd brood	60	3 [F] [G]	3	3 [h] [i]	F4-1 2009/5-6
Cross 08#2 2008/7/12	F2-1 blue[D]	F2-2 [e]	150	20	0	6	10 [m] [n] [o]	F3-2 2009/6-7
Cross 08#3 2008/7/4	F2-1 blue[D]	F2-2 [f]	170	20	2 [H] [I]	6	8 [p]	F3-3 2009/6-7
Cross 09#1 2009/6/2	F4-1 blue[F]	F4-1[h]	400	250	31	54	102	F5-1 2010/6-7
Cross 09#2 2009/6/10	F4-1 blue[F]	F3-1-2 [j]	120	60	4	17	26	F4-2 2010/6-7
Cross 09#3 2009/6/20	F4-1 blue[F]	F3-1-2 [k]	180	80	24 [J] f.*s.*	13	22	F4-3 2010/6-7
Cross 09#4 2009/6/6	F4-1 blue[G]	F4-1 [i]	140	70	30	0	29	F5-2* 2010/6-7
Cross 09#5 2009/6/16	F4-1 blue[G]	F3-1-2 [l]	60	20	3	3	10 [q] f.*s.*	F4-4 2010/6-7
Cross 09#6 2009/6/25	F4-1 blue[G]	F3-2 [m]	100	90	14 [K]	8	29 [r]	F4-5 2010/6-7
Cross 09#7 2009/6/26	F3-3 blue[H]	F3-3 [p]	290	60	8	11	17	F4-6 2010/6-7
Cross 09#8 2009/7/5	F3-3 blue[H]	F3-2 [n]	120	40	4	11	10	F4-7 2010/6-7
Cross 09#9 2009/7/5	F3-3 blue[I]	F3-2 [o]	100	30	4	7	10	F4-8 2010/6-7
Cross 10#1 2010/7/6	F4-3 blue[J] f.*s.*	F4-4 [q] f.*s.*	197	111	44 [L] [O] f.*s.*	0	25 [s] [v]	F5-3* f.*s.* 2011/6-7
Cross 10#2 2010/7/1	F4-5 blue[K]	F4-5 [r]	320	146	53 [M] [N]	0	46 [t] [u]	F5-4* 2011/6-7

* denotes pure blue line.
f.*s.*: forma *sugitanii*
[ ]: Alphabetical designation of individual adult used for crossing

Among the offspring of Cross 08#1, 6 larvae grew rapidly and achieved adulthood in September to November, 2008, without overwintering, producing 2 normal males and 4 females as part of the F3 generation (numbered as F3-1-1). One F3-1-1 normal male (identified as [E]) was coupled with an F3-1-1 female [g] on October 10 (Table 2).

Female [g] laid about 60 eggs in October, 2008. The larvae kept on growing without going into hibernation and were fed on potted *Celtis boninensis* placed indoors during the winter. Three blue males and 3 normal males emerged in May to June, 2009, as the F4 generation (F4-1). The remaining larvae of Cross 08 #1 completed a normal life cycle and yielded normal males in June, 2009, as part of the F3 generation (F3-1-2). Cross 08 #2 similarly produced normal males (F3-2), and Cross 08 #3 yielded both blue and normal males (F3-3). Further crossings were carried out in June to July, 2009, between 4 blue males (F4-1[F] and [G], F3-3[H] and [I]) and 9 females (F4-1[h] and [i], F3-1-2[j], [k], and [l], F3-2[m], [n], and [o], and F3-3[p]), i.e. 9 pairs (Crosses 09#1-9) in Table 2.

In June to July, 2010, both blue and purple males emerged from each of these crosses except for Cross 09#4, in which all 30 males were blue, suggesting conversion to a pure blue line at the F5 generation (numbered as F5-2). Unfortunately, this line failed to breed despite several pairing trials. In July, 2010, other pairings between 2 blue males and 2 females were performed (Crosses 10#1-2 in Table 2). In June to July, 2011, these two crosses produced

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Table 3. Results of successive breeding of *Sasakia charonda*.
Confirmation of pure blue line.

Cross Date paired Y/M/D	Phenotype of parents ♂ parent	♀parent	No. of larvae overwintered	No. of larvae used for experiment	Phenotype of offspring ♂blue	♂purple	♀	Generation with sequence number Emerged Y/M
Cross 11#1 2011/6/30	F5-3 blue[L] f.*s.*	F5-3 [s] f.*s.*	96	78	38	0	25	F6-1 * f.*s.* 2012/6-7
Cross 11#2 2011/7/9	F5-4 blue[M]	F5-4 [t]	310	198	91	0	79	F6-2 * 2012/6-7

* denotes pure blue line.
f.*s.*: forma *sugitanii*
[ ]: Alphabetical designation of individual adult used for crossing

44 and 53 blue males respectively, but no purples, again suggesting conversion to pure blue lines at the F5 generation (numbered as F5-3 and F5-4).

In order to confirm that this newly created blue line (F5-3,-4) was indeed pure, full-sib matings were carried out (Crosses 11#1-2 in Table 3). In June to July, 2012, these two crosses produced 38 and 91 blue males respectively, but no purples, in the F6 generation. Thus we could confirm that F5-3,-4 were indeed pure blue lines.

3. Crossing experiment between pure blue line and wild normal butterflies (Tables 4-5)

The pure blue lines and wild normal individuals were mated in the combinations shown as Crosses 11#3-6 in Table 4. In the next generation (F6), these crosses produced only normal purple males, 46 in total in June to July, 2012. This result suggests that the blue allele is recessive. Full-sib matings were then conducted in all combinations in July, 2012, as Crosses 12#1-4 in Table 4. These crosses resulted in 173, 66, 37, and 41 male progeny, respectively (Table 4). The respective segregation ratios between blues and purples were 1:3.21, 1:2.30, 1:3.62, and 1:2.72 (Table 5), and the observed proportions of blues were 0.236, 0.303, 0.216, and 0.268, respectively. Since all parents of the above 4 crosses were heterozygotes, an assumption that the blue allele is recessive leads to the following hypothetical mating results:

♂Aa x ♀Aa → AA + 2Aa + aa → 3 purples + 1 blue,

in which A: the dominant purple allele

a: the recessive blue allele

AA: homozygous purple

Aa: heterozygous purple

aa: homozygous blue.

Namely, the expected blue to purple ratio is 1:3, and the proportion of blues, 0.25 (1 in 4). The results of the experiments were statistically analyzed. We used a *Z*-test, in which standard normal distribution is used, to decide whether the observed proportions in Crosses12#1-4 are different from the expected proportion. The four observed segregation ratios were not different from the expected Mendelian segregation ratio ($|Z_0|$=0.425, 0.994, 0.478, and 0.266, p=0.67, 0.32, 0.63, and 0.79 respectively).

Discussion

The above breeding experiments confirmed the Mendelian recessive mode of transmission of the blue aberrancy. When initially encountered, this magnificent aberrancy was thought perhaps to be a sudden mutation, but when two blue males emerged from the same brood, the likelihood of a hereditary form, already existing in the population and probably recessive, was suspected. If so, it is impossible to know which females carry the blue allele based on external morphology. Mating a blue male to wild females to create presumed heterozygotes was the first step toward confirming the latter hypothesis. Repeated intra-generational couplings achieved a pure blue line in 2010, in the F5 generation, and two more pure lines in the same F5 generation in 2011. Using these latter two pure lines, additional crossing experiments between pure blues and wild normals confirmed the Mendelian recessive mode of transmission.

While breeding and accumulating blue specimens, we noticed subtle variations of blueness ranging from sky blue to dark blue, but a consistent difference of the blue from the normal purple color is evident in the distinct shining, metallic color visible in oblique view. This metallic color is derived from the ultrastructure of the blue scales. In an excellent work on the photonic crystal structure of wing scales in normal purple-colored *Sasakia charonda*, Matejkova-Plskova *et al.* (2009) found two types of scale, one of which has multiple layers of cuticle piled on the ridges, which are responsible for the iridescent color. A research group at the Osaka University Graduate School of Frontier Biosciences led by Dr S. Yoshioka has also

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Table 4. Results of successive breeding of *Sasakia charonda*.
Crossing experiment between pure blue line and wild normal butterflies.

Cross Y/M/D	Phenotype of parents		No. of larvae overwintered	No. of larvae used for experiment	Phenotype of offspring			Generation with sequence number Emerged Y/M
	♂ parent	♀parent			♂blue	♂purple	♀	
-	-	-	30*	30	0	13 [α] [β]	15 [γ] [δ]	F0 2011/6-7
Cross 11#3 2011/7/7	F5-4 blue[N]	wild [γ]	135	30	0	16 [P]	12 [w]	F6-3 2012/6-7
Cross 11#4 2011/7/6	F5-3 blue[O] f.s.	wild [δ]	81	40	0	15 [Q]	18 [x]	F6-4 2012/6-7
Cross 11#5 2011/7/12	wild purple[α]	blue line F5-4 [u]	80	30	0	11 [R]	7 [y]	F6-5 2012/6-7
Cross 11#6 2011/7/2	wild purple[β]	blue line F5-3 [v] f.*s.*	37	10	0	4 [S]	4 [z]	F6-6 2012/6-7
Cross 12#1 2012/7/12	F6-3 purple[P]	F6-3 [w]	439	404	41	132	130	F7-1 2013/6-7
Cross 12#2 2012/7/10	F6-4 purple[Q]	F6-4 [x]	245	220	20	46	64	F7-2 2013/6-7
Cross 12#3 2012/7/11	F6-5 purple[R]	F6-5 [y]	144	128	8	29	36	F7-3 2013/6-7
Cross 12#4 2012/7/15	F6-6 purple[S]	F6-6 [z]	151	102	11	30	25	F7-4 2013/6-7

*Wild overwintering larvae collected December 2010.
[ ]: Alphabetical designation of individual adult used for crossing
f.*s.*: forma *sugitanii*

Table 5. Results and statistical analysis of crossing experiment between pure blue line and wild normal butterflies.

	blue:purple ratio (blues:purples)	proportion of blues (p) (blues/total males)	Statistic (Z_0)	Testing $\lvert Z_0 \rvert < Z(0.05) = 1.96$
Hypothetical mating result	1 : 3	0.25 (p_0)	0	
Cross 12#1	1: 3.21 (41: 132)	0.236 (41/173)	-0.425	0.425 < 1.96
Cross 12#2	1: 2.30 (20 : 46)	0.303 (20/66)	0.994	0.994 < 1.96
Cross 12#3	1: 3.62 (8 : 29)	0.216 (8/37)	-0.478	0.478 < 1.96
Cross 12#4	1: 2.72 (11: 30)	0.268 (11/41)	0.266	0.266 < 1.96

been investigating structural color in *S. charonda*. An electron-microscopical comparison between blue scales and normal purple scales was presented by Mr S. Nagai, a member of Dr Yoshioka's group, at the meeting of the Lepidopterological Society of Japan Kinki Branch held in December, 2009. According to Nagai *et al.* (2009), the scales are composed of numerous parallel ridges. In the cross section each ridge has multiple attached folds, the distance between which is significantly wider (169 nm vs. 103 nm) in blue scales than in normal purple scales (unpublished data). Such spacing in periodic microstructure is known to be an important factor in cases where optical interference is the mechanism of structural color: a structure with a longer spacing distance reflects light with a longer wavelength. Thus, the difference in the distance between folds can explain the color difference between aberrant and normal types. We further speculate that the observed subtle variation in blueness is due to slight differences in the distance between folds of the scale ridges. Somewhat closer spacing might show up as a darker blue, and wider spacing might lead to sky blue (Dr S. Yoshioka, personal communication), although this requires electron-microscopic confirmation.

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We also noticed that " forma *sugitanii*", in which the hind wing's anal spot is white instead of red, and which is another example of recessive transmission as proven clearly by Ina (1989), can coexist with the blue aberrant form. F5-3 and F6-1 display both aberrations. In "forma *sugitanii*" there is a tendency for a less glistening and darker blue, but whether this tendency is just a reflection of the relatively small numbers of specimens obtained is unknown. These variations are shown in Figs 7-38.

As for the wider occurrence of the blue form of *Sasakia charonda*, similar aberrant forms have been rumoured to exist in northern Kyushu (Oita and Fukuoka Prefectures), the Kinki area (Kyoto Prefecture), and the Kanto area (Yamanashi Prefecture), although these have never been officially reported. The above mentioned specimen from Nagano Prefecture (Kodate *et al.*, 2009) is probably the same. There are some similar reports on the blue aberrancy. Sezai (2012) in Tochigi Prefecture reported 2 reared blue males originating from a single plant of *Celtis sinensis*, which may well signify a hereditary component. Also, Ueno and Takagi (2013) in Hokkaido reported 6 blue males captured from the same habitat in successive years, 2011 and 2012, when mass appearances of *S. charonda* were encountered. According to Mr H. Takagi (personal communication), in those years adult butterflies were so abundant that 500 to 600 adult males were easily captured each year, with specimens exhibiting the blue aberrancy comprising approximately 0.5% (6 in 1,000 to 1,200 in 2 years).

Since *S. charonda* has long been one of the most popular butterflies among amateur collectors in Japan, numerous larvae collected from the wild have been reared. Their rarity might be explained by an extremely thin and sporadic distribution. Only at the time of such massive emergences, such as occurred in Hokkaido in 2011 and 2012, might such a rare form have surfaced.

A characteristic feature of Hokkaido blue males is that the blue is much darker and less shining than in Shiga blue males, but quite similar to the specimen from Oita shown in Figs 5 and 6. Mr Takagi (pers. comm.) testified that the metallic blue of Hokkaido is less showy than the Shiga blue but that he can still easily distinguish it from the normal purple coloration by observing tilted specimens. We believe that all the mentioned aberrant specimens fall within the same spectrum of blue aberrancy. If examined by electron microscope, the Hokkaido blue form must have a slightly narrower fold distance than the Shiga blues along its scale ridges, but still far wider than normal.

Throughout Japan this blue form must be considered to be extremely rare and locally limited in distribution. Its conservation is paramount. For this reason, we are reluctant to disclose the precise location of its habitat in Shiga Prefecture. For the time being we are in the process of preserving the blue allele by continuing successive breeding.

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摘　要

メンデル式の劣性遺伝をすることが判明したオオムラサキの青い異常型（長﨑二三夫・山本毅也・北方健作・脇坂　昇）

はじめに

オオムラサキは，最も大きなタテハチョウの1種であり，日本，朝鮮，中国，台湾等に分布している．通常年1化で，幼虫越冬をする．翅表は黒褐色を基調に，♂は翅表の基部を広く紫色が被い，♀では紫色を欠く．日本のオオムラサキの♂の翅表については，紫色が少し薄い北海道から黒みを帯びる九州まで，微妙な地方変異が知られている．海外に目を転じると，台湾において青く輝くオオムラサキが知られており，また最近，長野県産の輝きの強い個体が提示されており（小舘，2009），これも青いオオムラサキの1型である可能性が高い．著者の一人は大分県北部から1頭の青みを帯びた輝きの強い♂を飼育により得ている（Figs 5, 6）．そんななかで滋賀県の同好者の間で（ブルー）と呼ばれているオオムラサキの存在を聞くに及んだ．同産地の越冬幼虫をたまたま累代飼育していたところ，青いオオムラサキの出現を記録し，遺伝形式を解明すべく累代実験を行うこととした．

最初の越冬幼虫は，2005年に滋賀県の鈴鹿山地より得たものである．飼育はそれぞれの著者の庭に植えてあるエノキを用い，ナイロンネットの袋掛けを行い，越冬幼虫を4月のエノキの芽吹きに合わせて放ち行った．成虫の羽化後♂は約10日経ち成熟してから，♀は羽化後2日目から7日目までを交配に用いた．交配はハンドペアリング法で行った．採卵はエノキの袋掛けネット内で行った．12月上旬に越冬幼虫が木を降りる頃を見計らい回収し，土を入れた植木鉢に保管した．

2005年12月に野外採集で得られた越冬幼虫からF2世代まで3世代累代して青い異常個体を得，その後出現した青い異常型個体から世代を重ねて純系作成を試みた．内交配を繰り返し，得られる翅色彩についてすべての組み合わせによるペアリングをして，純系の作成を目指した．一腹から得られた次世代の全個体が青色異常型となった後には，純系であることの確認のために純系個体同士の交配を行った．

青い異常型が出現する遺伝形式を見極めるために，青純系と新たに野外から得た幼虫より羽化した正常型との間で交配実験を計画した．2010年原産地より5 km離れた地点から得た30頭の正常型と青純系の2頭の♂，2頭の♀との間で4組の交配を行った．

飼育結果

1. 青い異常型個体の出現（Table 1）

Table 1の通り，2005年12月採集の同一産地に由来する正常型の1♂1♀を交配し，内交配による累代飼育の結果，2008年6月に3世代目として羽化した3頭の♂のうち2頭が青く輝く異常型であった（F2-1）．

2. 青の純系の作成の試み (Tables 2, 3)

累代を続けた結果，3世代後の組み合わせの一つから羽化した♂は，30頭すべてが青で，F 5世代で青の純系(*)が誕生したことが示唆された（Table 2: F5-2）．

これらが本当に純系であるかどうかの確認を行うため，純系と思われる2家族同士の交配を2011年6月から7月にかけて行った（Table 3: 11#1-2）．翌年2012年6月から7月にかけて，Cross 11#1, #2それぞれから38頭と91頭の青の♂のみが羽化し青の純系(*)であることが確認された（F6-1, 2）．

3. 青純系と野外の正常型との交配実験（Tables 4, 5）

前節で得られた青の純系♂と♀を，野外の正常型と交配させた（Table 4: 11#3-6）．これらの4組の組み合わせから，翌年2012年6月から7月にかけて，正常型の紫の♂46頭のみが羽化した（F6-3, 4, 5, 6）．青が1頭も羽化しなかったことより青は劣性であることが強く示唆され，羽化した♂，♀ともすべて青の遺伝子をヘテロの状態で保持していると考え

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られた. 最後に2012年7月に上記のF6-3, 4, 5, 6同士の間で交配を行った. すなわち青のヘテロ同士で交配を行ったことになる（Table 4: 12#1-4）. 2013年6月から7月にかけてCross12#1-4のそれぞれから青と紫の両方が羽化した (F7-1, 2, 3, 4).

上記の交配に用いた両親は, いずれも青に関してはヘテロでありまた青の遺伝子は劣性と想定していたので理論的に導かれる交配の結果は次のようになるはずである.

♂Aa x ♀Aa → AA + 2Aa +aa → 3紫 + 1青

ここでA: 優性の紫の遺伝子

a: 劣性の青の遺伝子

AA: ホモの紫

Aa: ヘテロの紫

aa: ホモの青

すなわち理論的には青対紫の分離比は1 : 3であり, 青出現率は0.25 (1/4) のはずである. そこで実験結果につき統計学的検討を行うこととした (Table 5). Cross12#1-4それぞれについて理論的な分離比から仮定される青出現率 (p_0) に対し実験で得られた比率 (p) が有意に異なるかを正規近似を用いて検定したところ, 4組ともメンデル遺伝の理論分離比で劣性遺伝すると判断された (Z検定 : Cross12#1-4それぞれ $|Z_0|$=0.425, p=0.67 ; $|Z_0|$=0.994, p=0.32 ; $|Z_0|$=0.478, p= 0.63 ; $|Z_0|$=0.266, p=0.79).

考　察

以上の飼育実験より青い異常型はメンデルの劣性遺伝をすることが確かめられた.

飼育実験を重ねるうちに集積された標本を検討すると, 青の青さにスカイブルーから比較的暗いダークブルーまで, 連続した微妙なバラツキのあることに気付いた. 青と正常型の決定的な違いは, 青は斜めに傾けて翅表を観察すると強く金属的に輝くことである. この金属色は構造色とよばれ鱗粉の微細構造に由来するものである. 正常型オオムラサキの鱗粉は大きく2種類に分けられ, そのうちの1種類が櫛状構造に外皮様のヒダが何層にも重なってついており, これが構造色を生み出しているという (Matejkov-Plskova *et al.*, 2009). 鱗粉の櫛状構造の櫛についている多数のヒダの間隔が正常型では103 nmに比し青では169 nmと広く, この広いことが光学的研究により, 青く輝く原因となっていることが突き止められている（永井ら, 2009）. この間隔が169 nmより少し狭いと, 濃い青へ, 広くなるとスカイブルーの方へバラツクのではないかと想像される（吉岡, 私信）. スギタニ型にも青い遺伝型が出現し, スギタニ型での青はギラツキ感がやや弱く, また青みが暗い傾向がある. これらの微妙な変異をまとめてFig. 7からFig. 38まで提示してみた. 連続した変異が理解されるものと思う.

青い異常型の存在は, 各地でささやかれてきた. 先に示した大分県をはじめとして, 福岡, 京都, 山梨からは報告は無いものの知られており, 栃木, 北海道からは最近報告されている. 栃木県の報告では, 同一のエノキから得られた8頭の越冬幼虫より青が2頭出現しており, 遺伝の関与を示唆している（瀬在, 2012）. 北海道では2011年と2012年のオオムラサキの大発生の年に, 計6頭の青い異常型が採集されたと報告しており（上野・高木, 2013）, それぞれの年に, 500～600頭のオオムラサキが容易に捕獲できたとのことである（高木, 私信）. そうであればおおざっぱであるが, 青の出現率は約0.5 %（6頭/600 × 2年）となり極めて稀であることが理解される. オオムラサキは、蝶の愛好家の間では最も人気ある蝶の一つであり, 過去半世紀以上数知れぬ越冬幼虫が飼育されてきた蝶である. にもかかわらず青い異常型の報告は, 上記のように最近の数例しかない. それほど稀な型ということであろう.

この青い異常型は極めて稀で, 分布が限られており, 保護が計られねばならない. その理由で詳しい産地の公表は控えさせていただいた. その一方で保護の目的で著者たちは累代の努力を継続中である.

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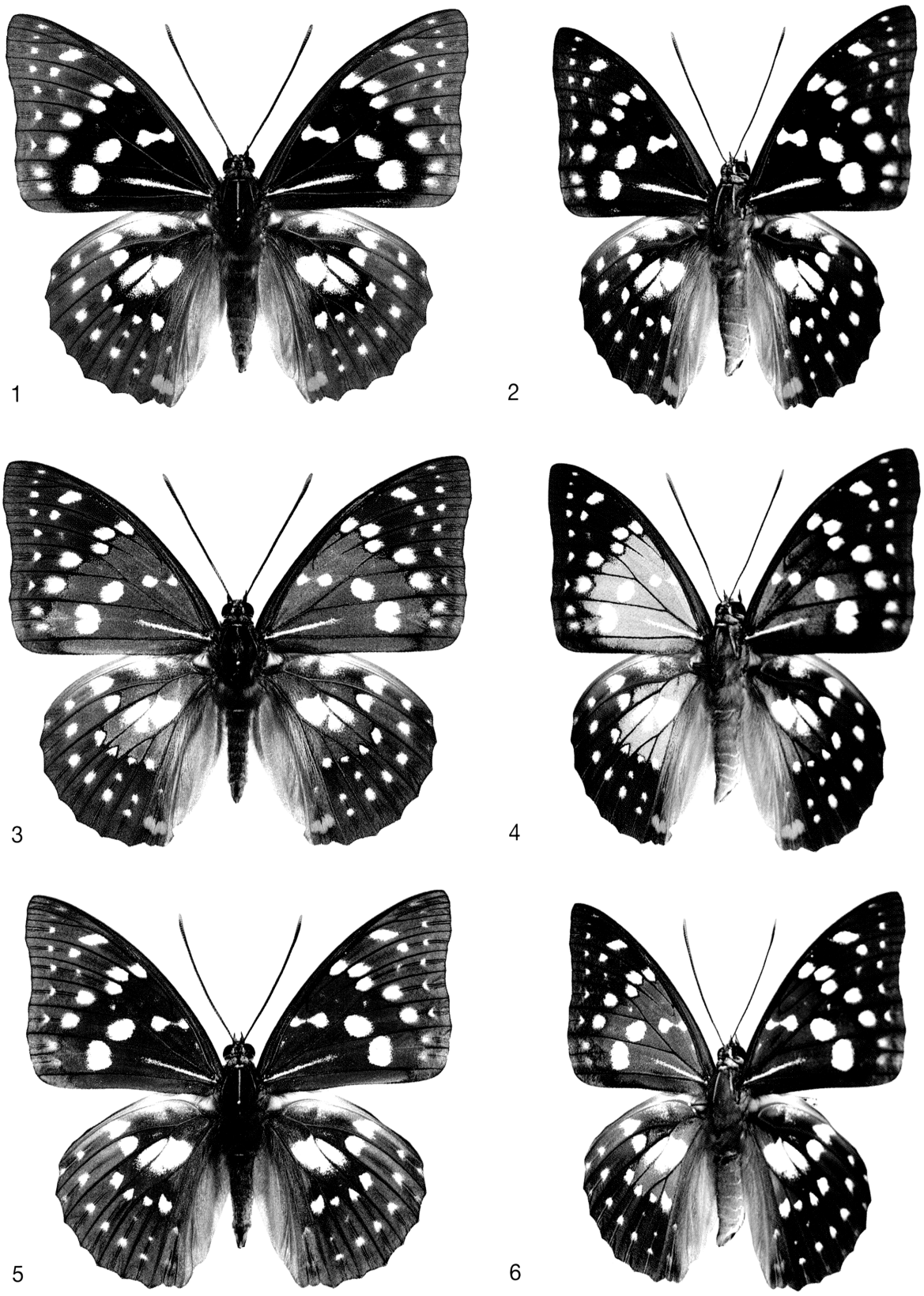

Figs 1-6. *Sasakia charonda*, males.
1-2: Shiga, normal, dorsal and 35° oblique view. 3-4: Shiga, blue aberrant form, dorsal and 35° oblique view.
5-6: Oita, blue aberrant form? dorsal and 35° oblique view.

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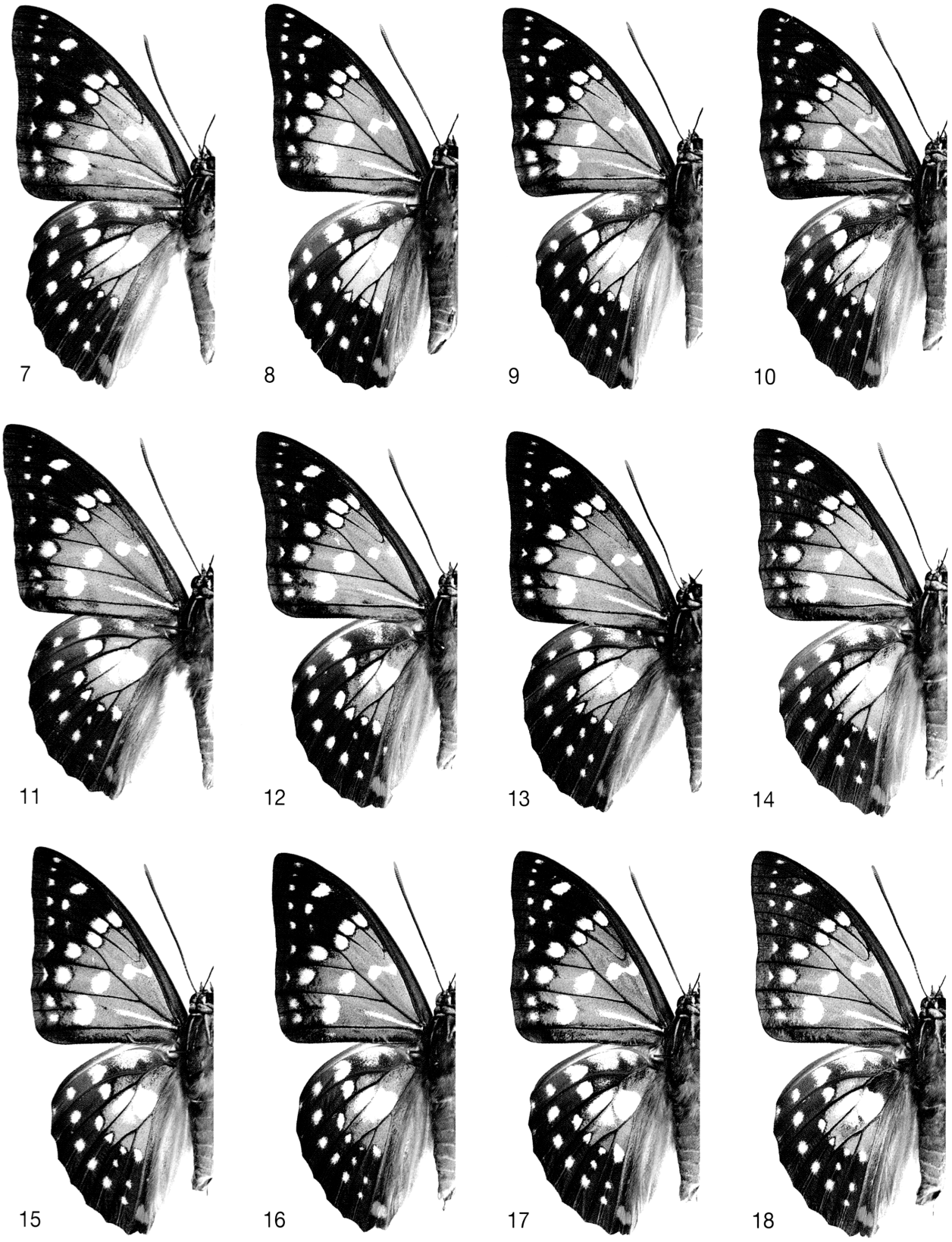

Figs 7-18. *Sasakia charonda*, males, Shiga, blue aberrant form, 35° oblique view, sky blue to intermediate blue.

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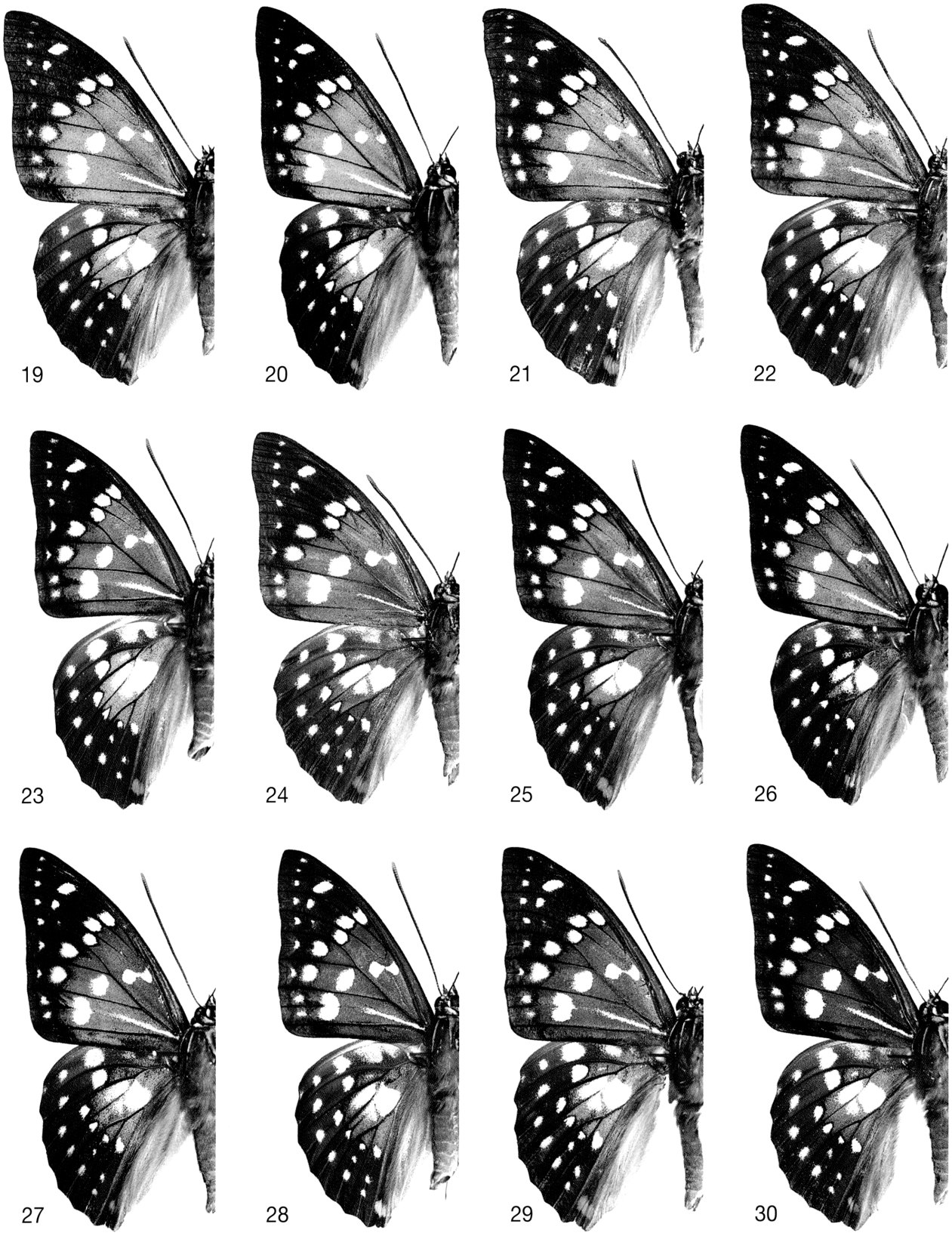

Figs 19-30. *Sasakia charonda*, males, Shiga, blue aberrant form, 35° oblique view, intermediate blue to dark blue.

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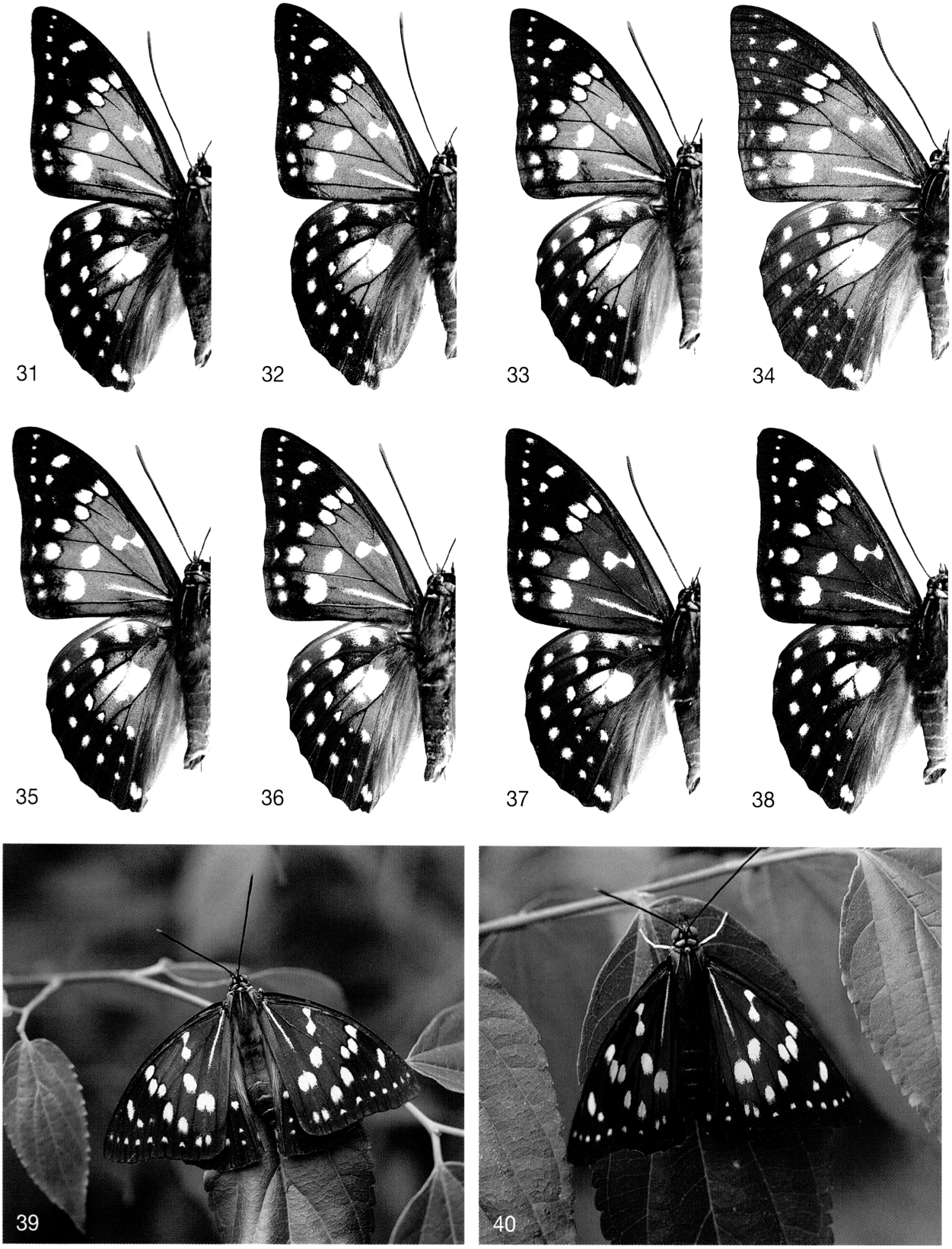

Figs 31-38. *Sasakia charonda*, males, forma *sugitanii*, Shiga, blue aberrant form, 35° oblique view, intermediate blue to dark blue.
Fig. 39. Blue aberrant form, emerged 2012/6/22.
Fig. 40. Blue aberrant form with forma *sugitanii*, emerged 2012/6/24.

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